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### Suggested Coating Procedures

Collaborative Biomedical Products Bovine Collagen, Type 1, may be gelled onto coverslips or tissue culture dishes, or used as a thin coating for cell attachment. Cells may be cultured on top of the gel, within the gel, or between gel layers.

Thin Coating- We recommend using collagen as a thin coating at 5-10 ug per cm<sup>2</sup>. Please use this as a guideline for determining the optimum concentration for your application.

- 1) Dilute material to 50 ug/ml using 0.01M HCl.
- 2) Add enough diluted material to coat dishes with 5-10 ug/cm<sup>2</sup>.

For example:

A 35mm dish has a surface area of approximately 10 cm<sup>2</sup>. One to two milliliters of the above solution would be sufficient to cover the dish.

- 3) Incubate at room temperature for one hour.
- 4) Carefully aspirate remaining solution.
- 5) Rinse well to remove acid, using PBS or serum free medium.
- 6) Plates may be used immediately or air dried. They may then be stored at 2-8°C for up to one week under sterile conditions.

### FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

**Use restriction for Europe and the United Kingdom:** This product may only be used as in *in-vitro* laboratory reagent. This product and its residue must not be allowed to come into contact with ruminating animals or swine.

Gelling Procedure- Bovine Collagen, Type 1 will gel when its pH is brought to neutrality using the procedure outlined below.

- 1) Prepare ammonia vapor chamber by taping a sterile 2 inch gauze sponge to the inside lid of a 150mm petri dish. Saturate the gauze with ammonium hydroxide. Place lid on 150mm dish and set aside.
- 2) Using aseptic technique, add sufficient volume of Bovine Collagen I to sterile glass or polystyrene culture dishes, spreading with sterile pipet to evenly cover entire growth surface. For dishes of 100 mm diameter add approximately 1.0 milliliter per dish; for 60 mm dishes add approximately 0.5 milliliter, and for 35 mm dishes approximately 0.2 milliliter.
- 3) Expose collagen coated dishes to ammonia vapor by placing the coated dishes with their lids off inside the 150 mm dish.
- 4) Expose for two minutes, and remove collagen dishes from chamber. Do not allow dishes to dry out at any point during this process.
- 5) Rinse dishes twice to remove the ammonium hydroxide, using phosphate buffered saline or sterile serum-free medium. Be careful not to dislodge the collagen coating.
- 6) Dishes are now ready for use.

An alternative to the ammonium hydroxide method follows:

- 1) Prepare neutralized isotonic collagen solution by mixing 8 parts chilled collagen solution to one part 0.01M NaOH and 1 part 10X phosphate buffered saline or 10X buffered serum-free cell culture medium.
- 2) Adjust the pH of the solution to  $7.4 \pm 0.2$  using 0.1 M HCl or 0.1M NaOH. Use either pH paper or phenol red to monitor the pH. Add the phenol red to the 10X PBS to a concentration of 5 ug/ml.
- 3) This diluted material may be used right away or stored at 2-8°C for several hours.
- 4) When ready for gelation, place desired amount of collagen in appropriate vessel and place at 37°C for 10-20 minutes. Collagen should gel within this time frame and is ready to use.

If you wish to prepare a fibrillar collagen gel:

- 1) Add neutralized collagen solution as prepared above to a thickness of 1.0-2.0mm.
- 2) Gel for 10-20 minutes at 37°C to promote gelation.
- 3) Leave dish uncovered in the laminar flow hood overnight or until dry.
- 4) Rinse remaining film with dH<sub>2</sub>O to remove excess salt and to rehydrate the collagen gel.
- 5) Plates may be used immediately or dried again and stored up to two weeks at 2-8°C.

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